SPORE-SHEDDING IN *GELIDIUM PUSILLUM* (GELIDIALES, RHODOPHYTA), WITH SOME OBSERVATION ON A MORPHOLOGICAL MUTANT

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ABSTRACT - Tetraspore shedding in *Gelidium pusillum* has been investigated in culture. The highest numbers of spores were shed between 12° and 22°C. No difference in spore liberation during 24 hours of light or darkness was found. The total number of spores produced per stichidium was estimated to be more than 5000, equivalent to 28 x 10^6 spores per gram plant (fresh weight). A temperature of 30°C for one week was lethal, but spores survived 2°C and total darkness for 2 weeks. An aberrant type of sporeling development was observed, resulting in globular plants. Back-crosses gave wild type tetrasporophytes and hybrid tetrasporelings developed into wild type and mutant morphotypes at a ratio of 1:1, indicating the presence of a single recessive mutation.

RÉSUMÉ - La libération des tétraspores chez *Gelidium pusillum* a été étudiée en culture. Le plus grand nombre de spores a été émis à des températures comprises entre 12° et 22°C. Une périodicité diurnale de libération de spores n'a pas été constatée. Le nombre de spores produites par stichidie a été estimé à plus de 5000, soit un total de 28 x 10^6 spores par gramme de plante (poids frais). 30°C pendant une semaine est léthal, mais les spores ont survécu à 2°C et en obscurité totale pendant 2 semaines. Un mutant, caractérisé par un développement aberrant, a été observé, produisant des plantes en pulvinule. Un croisement entre un type sauvage et un mutant a donné des tétrasporophytes de type sauvage dont les tétraspores se sont développées en deux différents morphotypes dans un rapport 1:1, indiquant une seule mutation récessive.

Key words - *Gelidium pusillum*, spore-shedding, morphological mutant, culture.

INTRODUCTION

Species of *Gelidium* produce tetrasporangia in special branchlets (stichidia), which makes it feasible to investigate the number of spores produced per stichidium under a variety of environmental conditions. Umamaheswara Rao & Kaliaperumal (1983) showed that dessication, light intensity, photoperiod, salinity and temperature all affected the shedding process in *Gelidium pusillum* (Stackhouse) Le Jolis from India, and the same authors demonstrated diurnal periodicity in spore-shedding with the maximum output of spores at night (Umamaheswara Rao & Kaliaperumal, 1987). Other studies have estimated the reproductive effort in *Gelidium* species in terms of the

number of spores produced per fresh weight (Suto, 1950; Guzmán-del Próo *et al.*, 1972; Kaliaperumal & Umamaheswara Rao, 1986). Most of these have been carried out on field-collected specimens brought into the laboratory. In the present study unialgal cultures of *Gelidium pusillum* were used to determine the number of spores produced per stichidium. The number of spore-shed were registrated under light and dark cycles and at various temperatures.

The characteristic spore germination pattern in species of Gelidiales has been studied repeatedly (see Santelices, 1988 for a review) and has also been used as a taxonomic character of the order (Papenfuss, 1966). An aberrant type of sporeling development was observed in some of the many germinating spores in this study that resulted in densely branched « globose » fronds. A male strain of this mutant was crossed with the wild type. The hybrid tetrasporophytes derived from the cross produced tetraspores and details of early sporeling morphogenesis in the two resulting morphotypes are described.

MATERIALS AND METHODS

The culture of *Gelidium pusillum* used was originally isolated from Wimereux (NW France). Culture methods were the same as in Fredriksen & Rueness (1990). Two young stichidia that had just started to shed spores (numbers 1 and 2) and two older ones that had already shed some tetraspores (number 3 and 4) were removed from the plant and placed in four separate wells in a multiwell plate, each containing 1.5 ml of medium at 17°C. The stichidia were kept in each well for 24 h either in continuous light (100 µmol photons m^{-2} s^{-1}) or in continuous darkness, after which they were transferred to a new well and the total number of spores shed was counted. The significance of differences between mean values was tested using a Student's t-test.

To determine the number of spores shed at various temperatures, five Petri dishes, each containing one stichidium, were placed at each of the following temperatures for 24 h: 27°, 22°, 18°, 12°, 8° and 2°C. The experiment started at the highest temperature, and the dishes were moved successively to lower temperatures after 24 h. Irradiance was 100 µmol photons m^{-2} s^{-1} under a 16:8 light: dark cycle. The average number of spores shed at each temperature was compared using a one-way ANOVA on log(x) transformed data (significance level 0.05).

The tolerance of spores to prolonged exposure (14 days) to extreme temperatures (2°, 27° and 30°C at 50 µmol photons m^{-2} s^{-1} in a 16:8h light:dark photoperiod) and high irradiances (250 µmol photons m^{-2} s^{-1} at 17°C in a 16:8h light: dark photoperiod) was determined. In a separate experiment exposure to complete darkness (17°C) and to continuous light (100 µmol photons m^{-2} s^{-1} at 17°C) was tested.

To study details of sporeling morphogenesis, tetraspores settled on cover slips were stained with Lactophenol-Wasserblau (Chroma) and mounted for microscopic examination.

RESULTS

The period between the start and the termination of spore shedding as determined in this study is at least 38 days, by which time each stichidium has released a total of 5000-6000 spores (Fig. 1). The number of stichidia produced on each tetrasporophyte was between 45 and 120 (n= 4, fresh weight of thalli 0.015-0.025g), i.e. up to 700 000 spores per plant or 28 x 10^6 spores per g fresh weight.

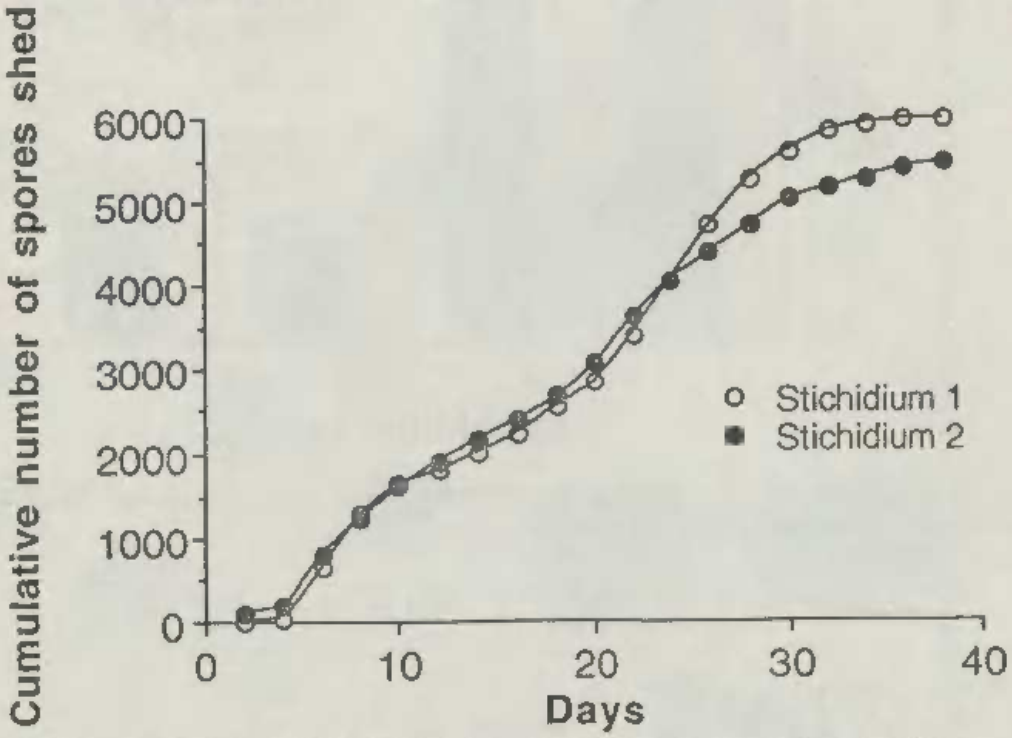


Fig. 1 - Cumulative number of *Gelidium pusillum* spores shed from two stichidia during a 38-day period.

There was no significant difference between the number of spores shed during 24 hours periods of darkness or light (Fig. 2).

The mean number of spores shed at different temperatures is shown in Fig. 3. The optimum temperature for spore shedding in this isolate of *G. pusillum* appears to be between 12° and 22°C, and the numbers of spores released were significantly lower at the other temperatures tested (Fig. 3 and Table I).

Table I - One-way ANOVA on log(x) transformed data for numbers of spores shed at different temperatures in *Gelidium pusillum*.

Source	df	Sum of squares	Mean square	F-test	P value
Between subjects	4	0.166	0.041	0.142	0.965
Within subjects	25	7.300	0.292		
Treatments	5	6.521	1.304	33.473	0.0001
Residual	20	0.779	0.039		
Total	29	7.466			

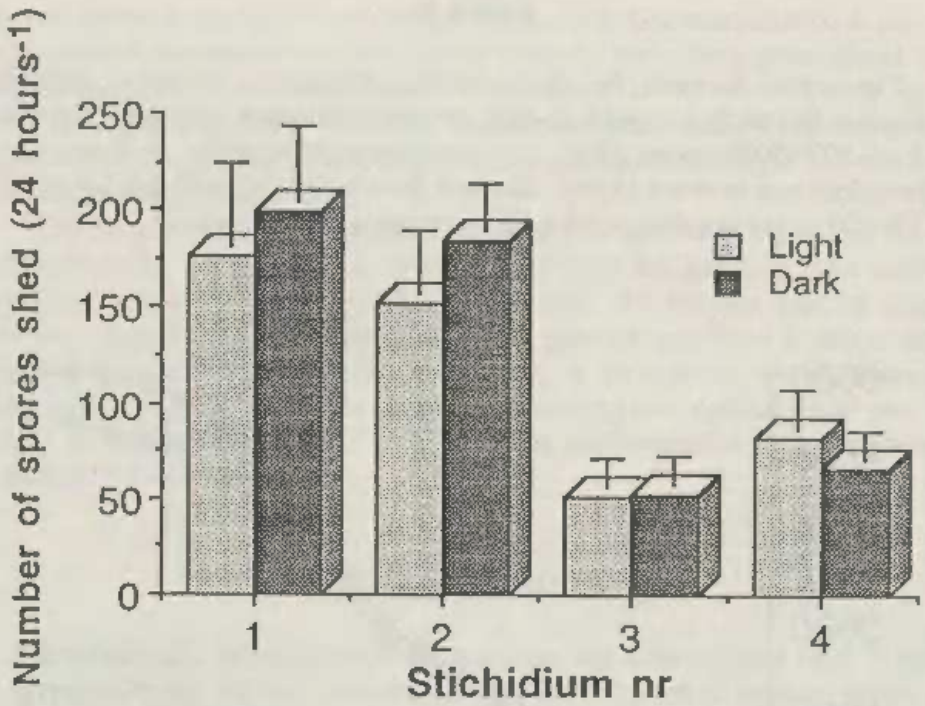


Fig. 2 - Average number of spores shed by four different stichidia of *Gelidium pusillum* during 24-hour periods of light and darkness. SE indicated.

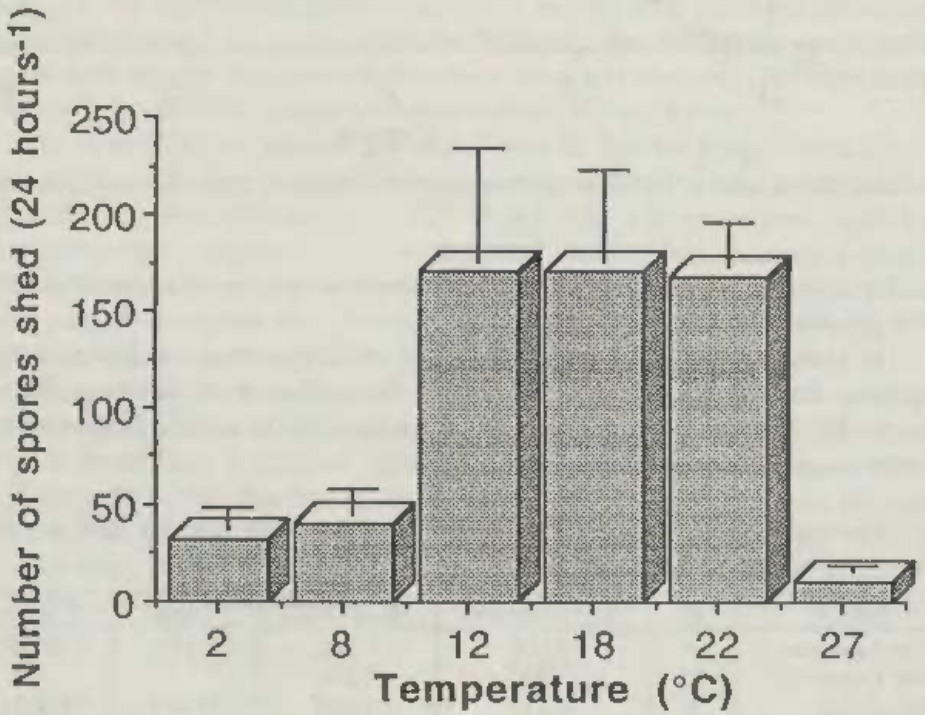
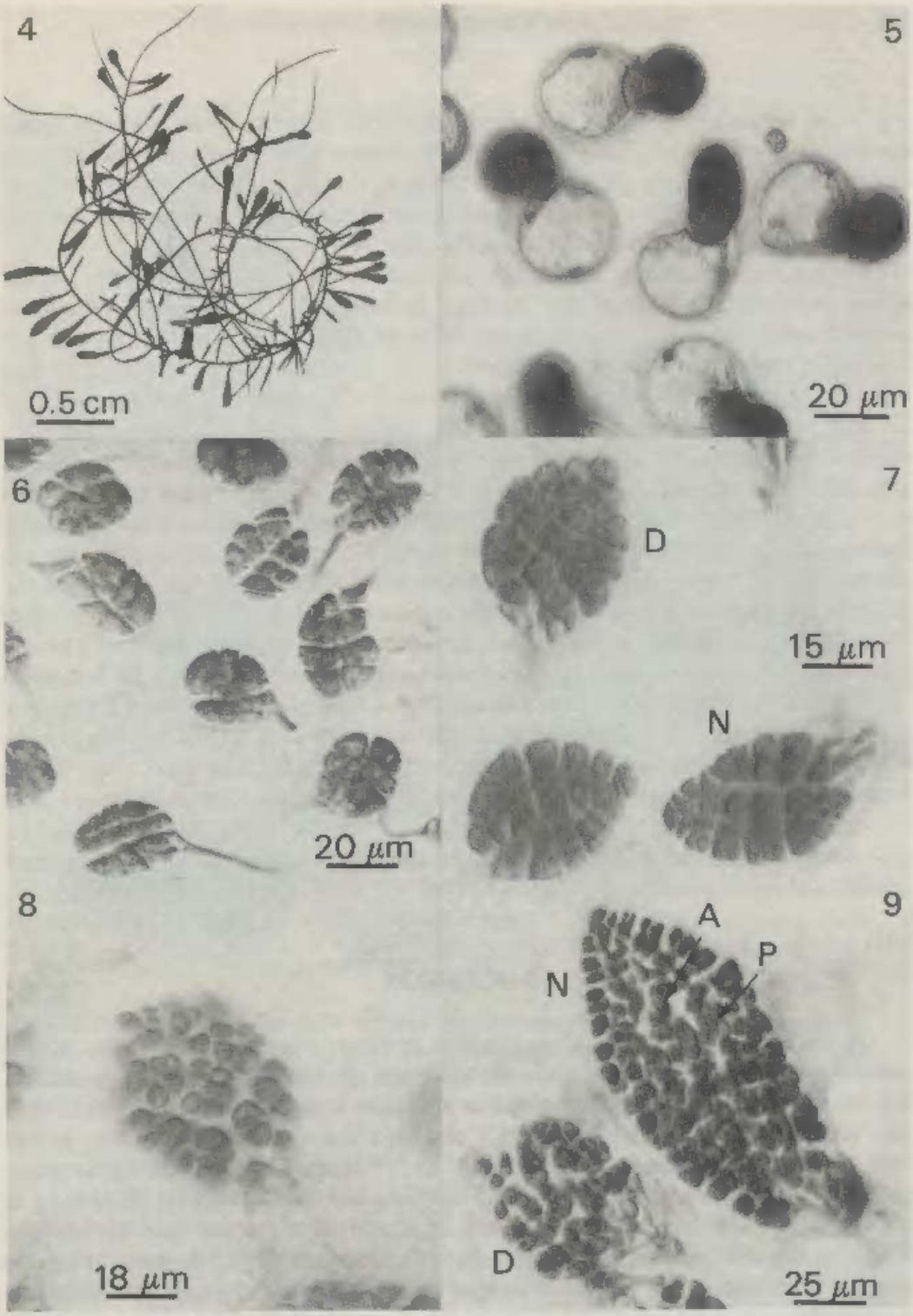


Fig. 3 - Average number of spores of *Gelidium pusillum* shed per 24 hours at different temperatures. SE indicated.



Figs. 4-9: *Gelidium pusillum*, differentiation into different morphotypes.
Fig. 4: Wild type tetrasporophyte of *Gelidium pusillum* with tetrasporangial stichidia. Fig. 5: Recently settled tetraspores in early stages of germination showing evacuation of spore contents into a germination tube. Fig. 6: Two-day-old tetrasporelings. Fig. 7: Four-day-old tetrasporelings. Two morphological types may be recognized by the shape of the apical cell. N = wild type with dome-shaped apical cell, D = mutant type with spherical apical cell. Fig. 8: Six-day-old mutant type sporeling. Fig. 9: Seven-day-old wild type (N) and mutant type sporeling (D). Note the central (A) and pericentral (P) cells in the wild type.

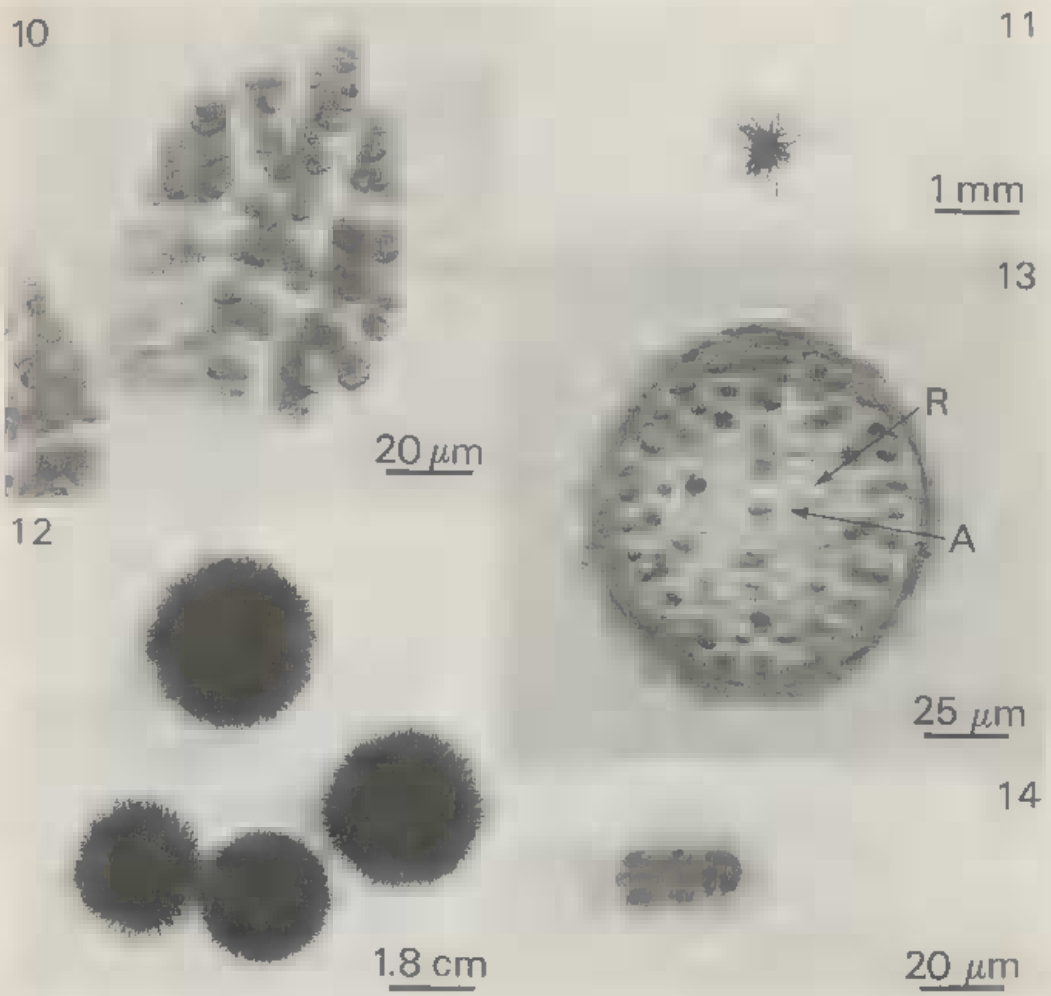
At temperature of 30°C, all spores were dead after one week, whereas spores survived temperatures of 2° and 27°C (at 50 µmol photons m^{-2} s^{-1}). Spores also survived high irradiance (250 µmol photons m^{-2} s^{-1}) and continuous light (100 µmol photons m^{-2} s^{-1}) at 17°C for 14 days. In complete darkness, the tetraspores underwent a few cell divisions and survived for about 14 days (Fig. 14).

A stichidium-bearing tetrasporophyte from culture is illustrated in Fig. 4, and spores germination typical of the Gelidiales is shown in Figs. 5 and 6. Among the hundreds of germinating tetraspores examined, an aberrant type was discovered and isolated in separate culture. One specimen of the mutant produced spermatangia and this was used in a back-cross with the «wild type» (term used for cultured specimens of *G. pusillum* with typical appearance). The resulting carpospores germinated into tetrasporophytes similar to the wild type, but following tetrasporogenesis, the hybrid tetraspores produced mutant (51.3%) and wild type (48.7%) progeny at a ratio of 1:1.

Two different morphological types could be distinguished about four days after tetraspore germination (Fig. 7). The wild type had a dome-shaped apical cell, whereas the mutant had a round apical apical cell. The round shape of the apical cell and the irregular arrangement of surface cells became more marked as the germlings grew older (Figs. 8, 10). The mutant was smaller and the inner axial and perixial cells were poorly defined (Fig. 9). The arrangement of second order filaments appears to be normal in older mutant plants (Fig. 13). After 2 months, the mutant plants were about 1 mm long and lacked the main axis characteristic of the wild type (Fig. 11). After one year in culture the mutant plants were globular and remained so for four years (Fig. 12). A transverse section of one axis of the mutant showed internal rhizoids in the centre (Fig. 13), similar to those seen in the wild type plant. Only male mutant plants have become reproductive, while the rest have remained vegetative. Among the wild type plants, females and males were found at a ratio of 1:1.

DISCUSSION

Each stichidium in *Gelidium pusillum* produces a large number of tetraspores and there are many stichidia on each plant. Studies of other *Gelidium* species indicate that the numbers of spores they shed are in the same range as found for *G. pusillum* in this study or rather lower. Suto (1950) observed that during the shedding season *Gelidium amansii* Lamouroux produced 10^4 to 10^5 spores per gram of thallus daily. Guzmán-del Próo *et al.* (1972) found that *Gelidium robustum* (Gardner) Hollenberg et Abbott reached a maximum of 27,453 tetraspores per month per thallus and Kaliaperumal & Umamaheswara Rao (1986) showed that *G. pusillum* could release about 1 x 10^6 spores per gram thallus over a period of about 2 years. For *Gelidiella acerosa* (Forsskål) Feldmann et Hamel, Screenivasa Rao (1971) found that tetraspore production was about 2 x 10^4 spores per plant per season. The number of tetraspores produced reflects a relatively high allocation of resources to reproduction. A large number of spores is important to ensure dispersal and recruitment. It is, however, important to remember that the number of spores produced by *Gelidium pusillum* in this study is probably close to the maximum possible since almost every branch is turned into a fertile stichidium.



Figs. 10-13: *Gelidium pusillum*, differentiation into different tetrasporeling morphotypes. Fig. 10: Nine-day-old mutant type. Fig. 11: Two-month-old mutant type. Fig. 12: Three-year-old mutant type. Fig. 13: Cross-section of ■ mutant as depicted in Fig. 12 showing central cell (A) and internal rhizoids (R). Fig. 14: Tetrasporeling after 12 days in total darkness.

In *G. amansii* from Japan, Suto (1950) found that the numbers of tetraspores shed reached a maximum in the afternoon. Suto (1950) stated that «accumulating effect of light is supposed to induce shedding». Umamaheswara Rao & Kaliaperumal (1987) found a definite rythm in the shedding of tetraspores in *G. pusillum*, with a peak output from 1800-2200 hours while Ngan & Price (1983) found that tetraspore output in *G. pusillum* from tropical Australia reached a maximum both in the morning (0500-0900) and in the afternoon (1300-1600). Spore output also reached a maximum in the morning in other species of *Gelidium* (Ngan & Price, 1983). In the present investigation no such peaks in shedding could be found after periods of light or darkness. However, one possible disadvantage of using cloned cultures such as these in experiments is that endogenous rhythms may be lost after several years in culture.

There was a significant temperature effect. Maximum shedding was found between 12° and 22°C. Both Katada (1955) (in *Gelidium amansii*) and Umamaheswara Rao & Kaliaperumal (1983) (in *G. pusillum*) found that the shedding increased with temperature up to about 25°C. Suto (1950) showed that shedding of tetraspores and carpospores in *Gelidium amansii* starts when the water temperature reaches 20° and 24°C, respectively. In *G. pusillum* 27°C is probably near the upper temperature tolerance limit since 30°C proved to be lethal.

In the present study tetraspores were exposed to various irradiance regimes and survived all except total darkness for longer than 14 days. Umamaheswara Rao & Kaliaperumal (1983) observed a reduction in spore output with increasing irradiance, suggesting that tetraspores are rather light-sensitive. However, the maximum irradiance used by Umamaheswara Rao & Kaliaperumal (1983) was 5500 lux (approximately 110 µmol photons m^{-2} s^{-1}, Lüning, 1981) which is not very high. At such levels of irradiances, it seems unlikely that the reduction in spore output with increasing irradiance can be explained by the sensitivity of newly-settled tetraspores to light.

Genetic segregation in red algae usually occurs in the haploid gametophytic phase, in which recessive mutations are not masked by wild type alleles. In this study, a back-cross using a mutant male and a wild type female produced wild type tetrasporophytes. During tetrasporogenesis a 1:1 segregation of the two types of tetraspores was seen, indicating the presence of a single recessive mutation. Van der Meer (1990) presented a survey of genetic studies that included an unidentified *Gelidium* species that produces a dwarf mutant plant. Harvey (1846) depicted a small globular specimen in his description of *Gelidium corneum* Lamouroux, suggesting that such forms occur occasionally in nature.

The morphological mutation in *Gelidium pusillum* described here was not sex-linked, as there were both female and male gametophytes among the wild type plants. Only male gametophytes (50% of the total) have been found to be fertile in the mutant plants; the rest have remained vegetative. These may be female plants in which a defective gene suppresses the ability to reproduce.

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